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Effect of application rates and abiotic factors on *Steinernema carpocapsae* for control of overwintering navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in pistachios

Joel P. Siegel [a,*], Lawrence A. Lacey [b], Bradley S. Higbee [c], Patricia Noble [a], Robert Fritts Jr. [d]

[a] *USDA/ARS, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA 93648, USA*
[b] *USDA/ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951, USA*
[c] *Paramount Farming Company, 33141 E. Lerdo Highway, Bakersfield, CA 93308-9767, USA*
[d] *Certis USA, 9145 Guilford Road, Suite 175, Columbia, MD 21046, USA*

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Abstract

The effect of reduced application rate, soil temperature at shallow depth ($\geqslant 2.5$ cm), and soil type on the efficacy of *Steinernema carpocapsae* against the navel orangeworm, *Amyelois transitella*, was evaluated in six field trials employing 1 m^2 plots conducted from November 2003 through December 2004 in Madera and Kern Counties, California. Nematodes were applied at a concentration of 100,000 infective juveniles (IJs)/m^2 (10^9/ha) in a volume of 187 ml water/m^2 (1870 L/ha) with a post-application irrigation in all trials. Mortality ranged from 7.9 to 64.9% in successful trials and percent reduction in live larvae per plot was as high as 74.6%. Percent reduction and mortality were highly correlated ($r^2 = 0.78$) and larval reduction typically was 10–11% greater than mortality for any treatment. In one trial, although nematode treatment significantly increased mortality compared to the controls, the treatment was deemed unsatisfactory because mortality was <15%. Soil temperature in this trial rose to 39 °C within 5 h after application. Nematodes failed in two other trials when soil temperature fell below freezing (minimum temperatures −3.0, −5.5 °C, respectively) several times in a 5-day period. We conclude that a commercially feasible application volume of 1870 L water/ha followed by post-application irrigation at this same rate was effective, and that soil maximum temperature at or below 32 °C during the first 24 h after application is necessary for treatment success. Published by Elsevier Inc.

Keywords: *Steinernema carpocapsae*; *Amyelois transitella*; Mortality; Pistachios; Soil temperature; Entomopathogenic nematodes

1. Introduction

The navel orangeworm (NOW), *Amyelois transitella* (Walker), is a key pest of pistachios in California. NOW larvae overwinter in nuts left after harvest, both on the ground and in trees (referred to as mummy nuts by orchard managers). They typically enter the pistachios from August through October and emerge from February through June. These larvae are currently controlled by cultural methods which include knocking the remaining nuts from the trees, blowing fallen nuts off the berm into the drive row and finally tilling the pistachios in the drive row into the soil (Bentley et al., 2000). Entomopathogenic nematodes (EPNs) are ideal agents to kill overwintering larvae in pistachios because they can enter nuts on the soil surface as well as shallowly buried nuts (<8 cm), and overwintering larvae are available for as long as 8 months. The efficacy of EPNs against NOW infesting almonds in trees was evaluated previously (Agudelo-Silva et al., 1987; Lindegren et al., 1987), but Siegel et al. (2004) were the first to demonstrate the ability of *Steinernema carpocapsae* (Weiser) to infect NOW infesting pistachios on the ground. We underestimated mortality in that study because fewer larvae (living + dead) were recovered in nematode-treated than

* Corresponding author. Fax: +1 559 596 2721.
E-mail address: jsiegel@fresno.ars.usda.gov (J.P. Siegel).

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control plots and living larvae were more likely to be recovered than cadavers. In our system, an alternate method such as analyzing the percent reduction in live larvae per treated plot may be more appropriate to assess the efficacy of EPNs.

Water is a limiting factor in many California orchards, and the application and post-application volume previously investigated by Siegel et al. (2004) is impractical (3740 L/ha) due to the amount of water used, the cost of water, and the cost of labor needed to apply these large volumes (labor cost may be as high as $62/ha). For example, an herbicide sprayer with a tank capacity of 1892 L can treat 4 ha with herbicide without refilling but cannot treat 1 ha with nematodes at an application volume of 3740 L/ha. Nematodes will only be used in this system if the amount of water and labor cost can be reduced. We had two goals in this study. Our first goal was to determine if infective juveniles (IJs) applied at the maximum commercially feasible volume combined with reduced post-application irrigation could reduce NOW. Our second goal was to determine the environmental conditions that affected treatment efficacy. In this paper we report the results of six small plot field trials, conducted from November 2003 through December 2004, that addressed the efficacy of an application volume of 1870 L/ha and the effect of abiotic factors on nematode efficacy (measured by both percent mortality and percent reduction).

2. Materials and methods

2.1. Source of nematodes and preparation

The IJs used in Trial 1 came from two sources. The liquid formulation, which was previously evaluated by Siegel et al. (2004), was produced by Certis USA (Columbia, MD) in a bioreactor located at Wasco, California. These nematodes were transported to the laboratory in continuously aerated containers (aquarium pump and air stone) and stored according to the producer's instructions at 10 °C until use. The clay formulation used in Trial 1 and the acrylamide gel formulation used exclusively in the remaining trials were produced by Becker Underwood (Ames, IA) and marketed by Certis USA as Millenium (Becker Underwood acquired the nematode assets of Certis USA in April 2005). This product was shipped chilled in a plastic tub containing 250 million IJs. The IJs in the tub were stored at 8 °C until used (no longer than 1 month) and were discarded when the expiration date was reached. In all trials, the nematodes were mixed with distilled water, counted, and the final suspension prepared on the day before application. This suspension was stored overnight at 10 °C in continuously aerated containers and 0.2 ml checked the next morning under a dissecting microscope for IJ movement to ensure that the nematodes were alive. If the nematodes were moving the suspension was deemed viable and the nematodes were transported to the field in continuously aerated containers and kept in shade (no more than 2 h) until application.

2.2. Nut infestation

All trials utilized culled nuts (hulled, partially dehydrated) received from a processing facility owned by Paramount Farms (Bakersfield, CA). Hulled nuts can be stored for long periods and support the development of NOW larvae. We noted no difference in nematode infectivity between infested hulled and intact pistachios and we previously used both types of pistachios in our experiments (Siegel et al., 2004). The nuts were infested with third instar larvae obtained from a laboratory colony maintained in our facility, and the pistachios were incubated at 22–24 °C for 3–7 days to allow the larvae to establish. The infested nuts were then stored at 10 °C to arrest larval development until used (maximum storage of 2 weeks). The nuts were removed and held at room temperature (22–24 °C) for 2 days before placement in the field. All nuts were thoroughly mixed the day before use and placed in 0.24 L containers (Sweetheart Cups Company, Chicago, IL) at a density of 100–150 nuts per container and one container was used per plot. The containers were randomized between blocks and treatments as an additional measure to ensure thorough mixing to eliminate bias. The average NOW prevalence was 15.8% in the trials and the majority (>95%) of the infested nuts contained a single larva.

2.3. Nematode application

Nematodes were applied at a concentration of 100,000 IJs/m^2 in all trials, using a CO_2 pressurized sprayer calibrated at 207 kPa (30 psi) and a hand-held, two nozzle spray boom equipped with TeeJet TP8010 nozzles (Spraying Systems, Wheaton, IL). The tip strainers were removed to reduce shearing of the nematodes. The nematodes were sprayed directly over the exposed nuts at a height of 40–60 cm. The control plots received 187 ml water followed approximately 15–20 min later by a post-application irrigation of 187 ml water. The plots received additional water in subsequent days from rainfall and/or when watering was scheduled for the orchard.

2.4. Study site description

The small plots (1-m^2) were placed on the north side of the berm in front of micro sprinklers with a flow rate of 22.7 L/h. The plots began at the third tree from the road to minimize edge effects and were placed consecutively under mature trees. The distribution of the treatments among the plots within a row was completely randomized and in all trials the pistachios were partially buried and were not covered by leaves. Five trials were conducted in Madera County at S&J Ranch (Trials 1, 2, 3, 5, and 6) and a single trial was conducted in Kern County at the Paramount Ranch at Belridge (Trial 4). The Madera County trials used two 16.2 ha blocks (Block 1: sandy loam soil; Block II: loamy sand soil) and the Kern County trial used one 16.2 ha block that had sandy loam soil. In Trial 1 the berm

was not pre-moistened but in the subsequent trials the berm was pre-moistened using micro sprinklers for 2 h before application and the micro sprinklers were run for 2 h post-application.

2.5. Experimental design

The number of treatments varied between trials but every treatment was replicated twice within a row. The pistachios were placed on soil-covered netting squares (625 cm^2) and then lightly sprinkled with soil. The plots were covered with nylon mesh screening (held in place with nails) 24 h after application to prevent removal of nuts by birds and mammals. The nuts were collected 7 days after treatment, placed in marked paper bags, transported and stored in plastic tubs, and held at 10 °C until they were cracked open and the larvae assessed. Trial 1, November 7–15, 2003, consisted of one control and three treatments per block with 10 replicates per treatment. The treatments were a liquid formulation of nematodes applied in volumes of 187 and 374 ml water/m^2, and a clay formulation of nematodes applied in a volume of 374 ml water/m^2. The post-application irrigation was 187 ml water/m^2 for all treatments. Trial 2, February 1–8, 2004, consisted of one control and two treatments per block with 10 replicates of each treatment per block. The treatments were applied in a volume of 374 or 187 ml water/m^2. Post-application irrigation was 187 ml water/m^2. Trial 3, March 11–19, 2004, consisted of one control and three treatments per block with 8 replicates of each treatment. The IJs were applied in a volume of 187 ml water/m^2 for all treatments and in one treatment the nuts were pre-irrigated with a volume of 187 ml water/m^2 instead of a post-application irrigation. Trial 4, March 18–26, 2004, consisted of one control and three treatments with 10 replicates of each treatment. The IJs were applied using volumes of 374 and 187 ml water/m^2 and in one treatment there was a pre-application irrigation volume of 93 ml water/m^2 and a post-application irrigation of 93 ml water/m^2; the other treatments used a post-application irrigation volume of 187 ml water/m^2. Trial-5, April 16–24, 2004, consisted of one control and two treatments per block with 10 replicates of each treatment. The IJs were applied at volumes of 187 and 467 ml water/m^2 and the 467 ml water/m^2 treatment did not receive a post-application irrigation. Trial 6, November 18–26, 2004, consisted of one control and two treatments per block with 10 replicates of each treatment. The IJs were applied at volumes of 187 and 93 ml water/m^2 with a post-application irrigation at the same volume.

2.6. Determination of percent mortality, percent reduction, and potential for nematode multiplication

All collected pistachios were cracked and examined for larvae within 2 weeks after collection; nuts that were not split (≈5%) were not included in the analysis because NOW could not infest them. Larvae were recorded as living if they moved when prodded. Larvae that were webbed outside the nuts or present in the soil surrounding the nuts were included in the analysis. Percent mortality was calculated as dead larvae per plot ÷ total number of larvae per plot. Percent reduction per block was calculated as total live larvae per treatment ÷ total split nuts recovered per treatment. This quotient was standardized as live larvae per 100 nuts and percent reduction calculated by comparing the values for control and treated plots. The potential for nematode multiplication in the field was determined in Trials 1–4 by placing recovered cadavers in covered petri plates containing moistened filter paper and then incubating the plates at room temperature (22–24 °C) for 14 days to monitor IJ emergence.

2.7. Temperature recording and soil moisture determination

Two HOBO data loggers (Onset Computer Corporation, Bourne, MA) were used to monitor soil temperature at a depth of 2.5 cm and two HOBO data loggers monitored air temperature at approximately 1.5 m above ground (placed on the underside of a branch) in the Madera County trials. The data for the two soil temperature data loggers were then averaged. A single data logger was used to monitor soil and a single data logger was used to monitor air temperature in the Kern County trial. Monitoring in all trials began 1 day before application and ended when the pistachios were collected.

Percent relative soil saturation was measured in Trial 2 using a Kelway HB-2 Soil Tester (Kel Instruments, Wyckoff, NJ). Percent relative saturation is a measure of a soil type's ability to hold water and is 100% when the soil type is at field capacity. In each block 28 readings were taken on the berm while 27 and 19 readings were taken on the drive row in blocks I and II, respectively.

2.8. Statistical analysis

Mortality data and differences in soil percent relative saturation were analyzed by two-way ANOVA and Fisher's protected LSD post hoc test. Reduction in live larvae per treatment was analyzed using multiple regression/correlation (mrc) analysis with orthogonal contrasts (Cohen and Cohen, 1983). Differences in total larval recovery between EPN and control treatments were analyzed using Relative Risk (R.R.), a statistic used in epidemiology to evaluate the likelihood of a specified dichotomous outcome (Kelsey et al., 1986). The relationship between percent mortality and percent reduction was analyzed by linear regression.

3. Results

3.1. Nematode emergence from cadavers

Emergence of EPN from cadavers held at room temperature was monitored in four trials and was greatest in the trials conducted when the daytime maximum soil temperature

did not exceed 32 °C and the nighttime minimum soil temperature was above 4 °C. Infective juveniles emerged from 58.2% ($n = 237$) of the cadavers in Trial 1, 0.1% ($n = 503$) of the cadavers in Trial 2, 20.6% ($n = 433$) of the cadavers in Trial 3, and 2.1% ($n = 96$) of the cadavers in Trial 4.

3.2. Differential recovery of larvae from control and treated plots

Living and dead larvae were more likely to be recovered from the control plots than the nematode-treated plots in Trials 1, 3, 4, and 5. Relative Risk (R.R.) was 1.78, 1.51, 1.19, and 1.20 ($P < 0.001$ in Trials 1, 3, and 4; $0.01 < P < 0.025$ in Trial 5) indicating that larvae were 78, 51, 19, and 20% more likely to be recovered from control plots. When the data from these trials were pooled (1669 larvae from 7531 nuts compared to 3556 larvae from 22,964 nuts) R.R. was 1.43, indicating that larvae were 43% more likely to be recovered from control plots ($P < 0.0001$). In the failed treatments (Trial 2, Trial 6) there was no difference in larval recovery between control and treated plots.

3.3. Mortality caused by nematodes

Application of nematodes significantly increased larval mortality in four trials (1, 3, 4, and 5). The greatest success occurred when the berm was premoistened with water from the micro sprinklers and a pre-application irrigation was as successful as a post-application irrigation. The three most successful trials (Trials 1, 3, and 5) occurred in Madera County ($F = 26.25$; $df = 3,63$; $P = 0.0001$; $F = 104.83$; $df = 3,53$; $P = 0.0001$; $F = 9.69$; $df = 2,53$; $P = 0.003$). In Trials 1 and 3, overall treatment mortality was significantly greater in Block II ($P = 0.0006$, 0.0001, respectively) and treatment efficacy differed between blocks ($P = 0.03$, 0.02, respectively), Table 1. In Trial 1, although the liquid

Table 1
Successful small plot (1 m^2) trials with *Steinernema carpocapsae* for the control of *Amyelois transitella*

Formulation	Application rate	Pre-application irrigation	Post-application irrigation	Percent mortality (±SD)[a]	Percent reduction[b]	Larvae per 100 nuts[b]	Total larvae	Total nuts
Trial 1, Block I								
Control	1870	0	1870	5.8 ± 8.7 a	—	18.8 a	291	1474
Liquid	1870	0	1870	13.2 ± 7.6 b	25.4 b	14.0 b	230	1476
Clay	3740	0	1870	19.6 ± 8.7 b	31.7 b	12.8 b	238	1496
Liquid	3740	0	1870	31.1 ± 17.4 c	43.5 c	10.6 c	226	1450
Trial I, Block II								
Control	1870	0	1870	5.0 ± 5.2 a	—	12.4 a	191	1460
Clay	3740	0	1870	24.8 ± 10.1 b	43.5 b	7.0 b	133	1470
Liquid	1870	0	1870	34.5 ± 18.9 bc	50.4 bc	6.2 bc	113	1300
Liquid	3740	0	1870	45.8 ± 11.3 c	59.4 c	5.0 c	128	1452
Trial 3, Block I								
Control	1870	0	1870	0.9 ± 1.7 a	—	38.3 a	296	767
Gel	3740	0	1870	42.8 ± 10.1 b	65.3 b	13.3 b	178	767
Gel	1870	0	1870	41.7 ± 13.3 b	67.0 b	12.7 b	161	751
Gel	1870	1870	0	47.4 ± 15.8 b	67.7 b	12.4 b	173	776
Trial 3, Block II								
Control	1870	0	1870	2.5 ± 3.0 a	—	26.7 a	219	801
Gel	3740	0	1870	52.5 ± 11.8 b	59.1 b	10.9 b	153	667
Gel	1870	0	1870	64.9 ± 4.9 c	74.6 c	6.8 c	130	676
Gel	1870	1870	0	64.6 ± 8.2 c	70.4 c	7.9 c	148	683
Trial 4								
Control	1870	0	1870	1.5 ± 2.2 a	—	37.9 a	407	1056
Gel	3740	0	1870	8.4 ± 7.9 b	12.3 b	33.2 b	383	1057
Gel	1870	0	1870	7.9 ± 5.0 b	15.3 b	32.1 b	372	1069
Gel	1870	935	935	12.3 ± 11.0 b	10.3 b	34.0 b	375	1072
Trial 5, Block I								
Control	1870	0	1870	22.6 ± 42.0 a	—	9.3 a	124	1031
Gel	1870	0	1870	34.5 ± 47.8 ab	42.9	5.3 b	97	1015
Gel	4670	0	0	39.3 ± 49.0 b	0	10.6 a	178	1017
Trial 5, Block II								
Control	1870	0	1870	19.9 ± 40.0 a	—	10.8a	141	1040
Gel	1870	0	1870	54.0 ± 50.2 b	71.1a	3.1 b	63	931
Gel	4670	0	0	50.5 ± 50.3 b	53.4 b	5.0 c	91	917

Nematode application rate, pre-application, and post-application irrigation rates are liters per hectare. The nematode concentration was one billion IJs per hectare.

[a] Means followed by a different letter within a trial are significantly different at $P < 0.05$ (Fisher's protected LSD).

[b] Means followed by a different letter within a trial are significantly different at $P < 0.05$ (t test orthogonal comparison).

formulation applied at 187 ml was ineffective in Block I, in Block II it outperformed the clay formulation and was equally as effective as the liquid formulation delivered at twice the volume. In Trial 3, there was no difference among the treatments in Block I while in Block II mortality was greater in the treatments with the lowest application volume ($P = 0.006$). In contrast to this pattern of uneven efficacy between blocks, all treatments in Trial 5 were equally successful in both blocks. In Trial 4 (Kern County) although mortality in the treated plots was significantly greater than mortality in control plots ($F = 3.72$; $df = 3,36$; $P = 0.02$), it was substantially lower than in the other successful trials (7.9–12.3%). In the two failed trials in Madera County, overall mortality was 13.3% (799 total larvae) in Trial 2 and was 14.8% (305 total larvae) in Trial 6. This mortality was comparable to the control mortality observed in the other trials.

3.4. Percent reduction caused by nematodes

Percent reduction of live larvae followed the same pattern as percent mortality, except that the impact of nematodes was greater for almost every treatment (Table 1). In Trial 5, there was a clear-cut difference in nematode efficacy between blocks that was not apparent when two-way ANOVA analysis was conducted using mortality as the outcome variable. This difference between blocks was most pronounced in the control and high volume plots ($t = 2.26$; $P = 0.02$, $t = 4.81$; $P < 0.0001$, respectively) and underscored the previous observation that treatments were the most effective in Block II.

3.5. Relationship between percent mortality and percent reduction

A total of 3770 larvae were recovered in the successful trials, and in these experiments percent mortality and percent reduction were highly correlated ($r^2 = 0.78$). Their linear relationship is described by the equation $Y = 0.101 + 1.017\ X$, where Y is percent reduction and X is percent mortality ($F = 52.68$, $df = 1,16$; $P < 0.0001$) (Fig. 1).

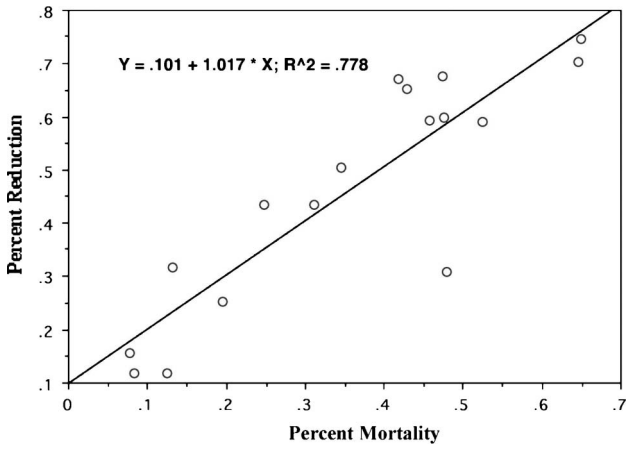

Fig. 1. The relationship between percentage larval mortality and percentage reduction in live larvae recovered per treatment ($Y = 0.101 + 1.017\ X$, $r^2 = 0.78$, $P < 0.0001$).

Table 2
Soil temperature at a depth of 2.5 cm for the 7 days following nematode application

Trial	Mean temperature for the interval (°C)	Maximum (°C)	Minimum (°C)
1	11.2	31.8	−1.4
2[a]	8.3	31.3	−2.7
3	19.4	48.8	6.0
4	21.1	42.0	7.1
5	15.4	42.0	3.0
6[a]	7.9	26.8	−3.2

[a] Failed treatment.

Percent reduction was designated as the dependent variable because it is determined by mortality. Percent reduction is 10–11% higher than mortality; the maximum mortality of 65% observed corresponded to a 76% reduction in live larvae.

3.6. Soil temperature and soil moisture

The mean, maximum and minimum soil temperatures for each trial are reported in Table 2. High temperature was most deleterious in Trial 4, when it exceeded 39 °C for 5 h after application (Fig. 2). Trial 3 had the highest soil temperature recorded, and at 48 h post-application its soil temperature was essentially the same as in Trial 4, but the temperature during the first 24 h after application did not exceed 32 °C. Likewise, in Trial 5 soil temperature did not exceed 25 °C during the first 48 h but rose to a maximum temperature of 42 °C afterwards. Minimum soil temperatures were lowest in Trials 1, 2, and 6, and in the two failed trials (Trials 2 and 6), nighttime minimum temperatures were −2.7 and −3.2 °C, respectively, whereas the mean soil temperatures for the 7-day period were 8.3 and 7.9 °C. In Trial 6, soil temperature remained below 10 °C for the first 48 h and temperatures fell below freezing from days 3–7 post-application.

Overall, soil moisture in the drive row was almost three times greater than the berm (28.4 ± 11.4 vs. $9.9 \pm 8.1\%$, $P = 0.0001$) and the soil in Block I was 1.5 times as moist as the soil in Block II ($22.7\% \pm 13.2$, $15.4\% \pm 12.9$, $P = 0.0001$). The moisture level on the berm increased after irrigation to $57.2\% \pm 13.9$ and $41.3\% \pm 7.3$ in Blocks I and II, but Block I was still more moist ($t = 8.3$; $df = 34$; $P < 0.0001$) than Block II.

4. Discussion

Our first goal in this study was to evaluate the effect of reduced application volume on nematode efficacy. Although we obtained significant mortality at the reduced application volume, it was not at the level attained in our previous study ($\geqslant 94\%$). When we used a more appropriate measure to evaluate treatment efficacy, percent reduction in live larvae per plot, nematode application at the reduced rate produced as much as a 60–75% larval reduction. It is the standard practice in turf management to follow a nematode application

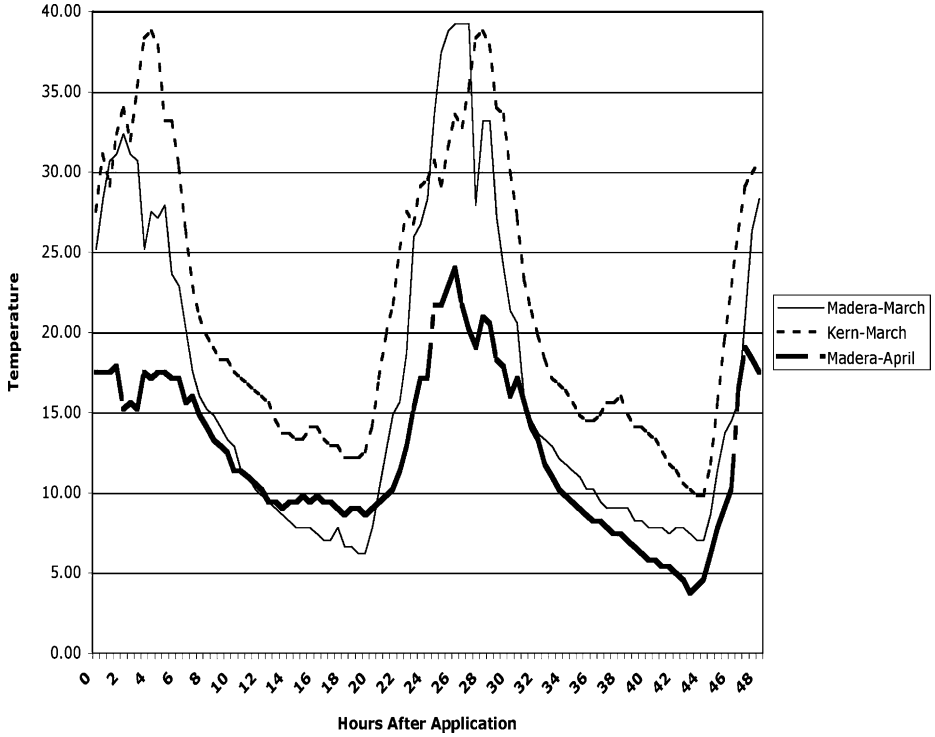

Fig. 2. Soil temperature (°C) during the 48 h after application for March treatments in Madera (Trial 3) and Kern (Trial 4) Counties, and April treatment in Madera County (Trial 5).

with post-application irrigation and we found no reason to deviate from this procedure in pistachios. Our best results (Trials 3 and 5) were obtained when the berm was pre-moistened and the nematode application was followed by a post-application irrigation. We do not feel that it is productive to evaluate lower application rates because of the rapidity of evaporation at low humidity, but it may be possible to further manipulate the length of time the micro sprinklers are run. Our emphasis in this and our previous study was the berm, which extends 91–121 cm outward from the tree-trunks and contains no vegetation, because in many orchards this is where most of the pistachios fall. In our study sites, the berm was 26% of the total area within the orchard and a single hectare of treated berm would comprise 3.85 ha of orchard. If the berm is targeted, there are fewer nematodes needed and the amount of water and labor is reduced. It may not be economical to treat orchards when the entire area is covered with pistachios and must be treated.

Our second goal in this study was field assessment of nematode treatments over a range of soil temperatures in order to establish the upper and lower limit for application. At the shallow depth evaluated in our NOW-pistachio system, soil had no buffering effect and air temperature was nearly identical to soil temperature. Soil temperature fluctuated daily and the range was as large as 42 °C in March. At first glance our data appear erroneous because soil temperatures were higher in March than April. However, in the Central Valley in 2004, March was warmer than April (air temperature >38 °C on several days in March) and the berm in March also received direct sunlight (trees were leafless), while in April the berm was shaded. Previous researchers reported that the upper limit for *S. carpocapsae* infectivity is 28–32 °C (Gouge et al., 1999; Grewal, 2002) and the upper limit reported for survival of previously conditioned IJs is even higher, 40 °C (Jagdale and Gordon, 1997, 1998). In our study nematodes were the most vulnerable to temperatures greater than 32 °C during the first 24 h after application and treatments were still successful when soil temperature subsequently rose as high as 48.8 °C. It is the timing of exposure to high temperature that is paramount and despite these high temperatures nematodes still successfully emerged from the cadavers that were left in the field one week and then returned to develop in the laboratory. At the other end of the spectrum, the low temperature infectivity threshold reported for *S. carpocapsae* is 10 °C (Gouge et al., 1999; Jagdale and Gordon, 1997, 1998), but IJs inside cadavers survived temperatures of −8 °C for 24 h (Lewis and Shapiro-Ilan, 2002). In our study performance in the field was erratic when temperature fell below freezing and insufficient moisture on the berm in Trial 1 may have contributed to the reduction in treatment efficacy noted. Previously, nematode efficacy was also reduced when the minimum temperature fell to −1.5 °C (Siegel et al., 2004). Mean soil temperature may be a better predictor of treatment success because in the two failed trials, mean temperature was below the infectivity threshold of 10 °C (Table 2). We conclude that although there was strong evidence for a 24 h window of vulnerability to temperature greater than 32 °C, we cannot establish a similar time period for vulnerability to temperatures at or below freezing.

Soil type and moisture undoubtedly modify the effects of temperature. Kung et al. (1990) reported that *S. carpocapsae* survived better on sandy loam soil than clay and sand but did not evaluate loamy sand, which in our study was the soil type where nematodes were most effective. The possible effect of soil type was confounded with moisture and soil heating differences. Kung et al. (1991) reported that in the laboratory optimal survival of *S. carpocapsae* occurred on soil with a moisture level of 2%, and if this occurred in the field the drier soil in Block II may have increased treatment efficacy. The magnitude of the temperature fluctuation was also greater in Block II in 4/5 trials. Further study is warranted to determine if nematode performance is truly better on loamy sand than sandy loam soil.

In our previous study (Siegel et al., 2004), we reported that more larvae were recovered in control than treated plots and the results in this study were similar. There are several possible explanations for this difference that are not mutually exclusive. If preferential removal of cadavers by ants occurred in treated plots, then larval recovery would be reduced and in fact larval recovery was significantly lower ($P < 0.0001$) in the block with the greatest ant activity. Larvae in treated plots may be more likely to abandon nuts and possibly even leave the plots to avoid nematodes, which would increase their exposure to predators and adverse environmental factors. Finally, we may be more efficient at recovering living than dead larvae in rotten nuts. Regardless of the reason, if percent reduction of live larvae is used as the outcome variable, this bias is eliminated.

In conclusion, a concentration of one billion *S. carpocapsae* IJs/ha applied in a volume of 1870 L/ha followed by post-application irrigation at that same volume to pre-moistened plots consistently reduced NOW. There is a 24-h window post-application for nematode vulnerability to soil temperature greater than 32 °C but we could not determine a similar time window for post-application exposure to freezing temperatures. Lewis and Shapiro-Ilan (2002) reported that in the laboratory nematodes were most vulnerable to freezing 48–72 h after infection and based on their data and this study we conclude that the success of a field application can be determined within the first 3 days. Environmental factors played a role in nematode efficacy because if mortality was solely dependent on initial deposition of nematodes, there would be no difference in efficacy between blocks that received the same water volume delivered by the same sprayers. Although we demonstrated that nematodes were effective at a practical water volume, ultimately it is the total cost of this treatment that will determine its adoption. Further research is necessary to determine if the increased use of micro sprinklers can offset the amount of water and labor used for post-application irrigation, as well as the feasibility of other large-scale application methods that will minimize total cost and maximize treatment success.

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